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Expand 4 cultures from Jones from 1/2 ml to 10 ml 37°C shaker
to make glycerol stocks of all 4 grow 1 clone at CN# 2 300 ml

CN88 clone 1 18.68.1	200 ml culture	} LB
CN88 clone 2 18.68.3	10 ml Min	
CN89 clone 1 18.68.2	200 ml culture	
CN89 clone 2 18.68.4	10 ml Min	

CN 88 = 6kb PSE from CDI in BS⁺ as Hind III Xba I @ 5' end of PSE

CN 89 = 6kb PSE from CDI in BS⁺ as Hind III Sac I @ 5' end of PSE

Quagen Minicolumn CN88 clone 1 & CN89 clone 1 16p 20, 2 min, QBT 1x30 ml, QC 2x24-120
10 ml Minicolumn prep alk lysis CN88 clone 2 CN89 clone 2

Tube	DNA	Wet	10x NEB Buffer	10x BSA	Alt. C	Enzyme
1	CN88 clone 1		2	1 ul		-
2	"					Hind III
3	"					Xba I
34	CN88 clone 2					-
5	"					Hind III
6	"					Xba I
7	CN89 clone 1					-
8	"					Hind III
9	"					Xba I
10	CN89 clone 2					-
11	"					Hind III
12	"					Xba I

see to cloning
this
CN88 clone 1 is good

Transform into DH5⁺ cells to grow DNA for stock

BHG-106-H 16.192
BHG-116-H 16.192
CN84.1 18.63

Make SB Media for BHG cultures

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Witnessed & Understood by me,

H. Lam pms k2

Date

5/16/95

Invented by

Recorded by

[Signature]

Date

7/8/95

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Restriction digest

Lane	DNA	6cl	10X NBS Buffer	10X PSA	disto	Enzyme
1	CN78	7ul ✓	1 ✓	0	2, 1 ✓	+
2			1 ✓		1 ✓	NarI ✓
3			2			HindIII ✓ linear
4			EcoRI ✓			EcoRI ✓ linear
5			✓			BglII ✓ linear
6			✓		0	EcoRI/BglII ✓ 3200, 6409
7			✓		0	EcoRI/BglII/HindIII ✓ 900, 2300, 6409
8	CN85	2ul ✓	2 ✓	1ul ✓	5 ✓	+
9					5 ✓	ClaI ✓ 2,760, 7500
10						XbaI ✓
11						ClaI/XbaI ✓ 158, 2725, 6409
12	BHG-83-1	4ul ✓	2 ✓	0	4 ✓	+
13	"				4 ✓	HindIII ✓
14	BHG-83-2	1ul ✓			7 ✓	+
15	"				7 ✓	HindIII ✓ to 315

CN78 looks fine
 CN85 looks fine
 BHG-83's must have forgotten enzyme cleavage



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Witnessed & Understood by me,

H. Lamparky

Date

5/16/95

Invented by

5/16/95

Date

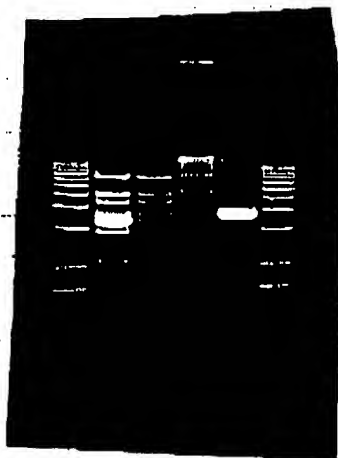
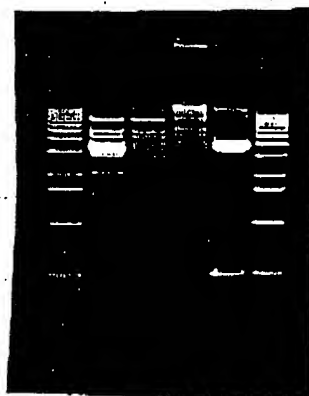
5/16/95

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Tube DNase vol Ex PCR CHC Enzyme

A BHG 10 20.4 1ul ✓ 2 0 7ul ✓ Hind III ✓
 B BHG 11 20.4 1 ✓ 1 1 7 ✓ Hind III ✓
 C BHG E3 1 20.1 8 ✓ 1 1 0 Hind III ✓
 D BHG E3 2 20.1 1 ✓ 1 1 7 ✓ Hind III ✓

1 CN 76 2ul ✓ 2 1ul 5ul ✓ 0 Supercut
 2 16.192 2 2 1ul 1ul ✓ Hind III ✓ lensin
 3 1.20 1 1 1ul 1ul ✓ Sac I 16kb, 5'80
 4 1 2 2 1ul 1ul ✓ Xba I 3880, 2986
 5 1 2 2 1ul 1ul ✓ Cla I 1156, 5744 To 10-11



BHG 10 still looks good the large band
 at 3+Kb is too large
 BHG 11 looks good
 BHG E3-1 has the correct bands but is
 smearing!
 BHG E3-2 is trash



CN 76 looks fine!
 Need OD & pH in 1ml purple rock -20°C chest.

CN 76 16.192 OD 26.0 2580 264/280 1.8 4 mg/ml

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M. Lamparky

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5/10/95

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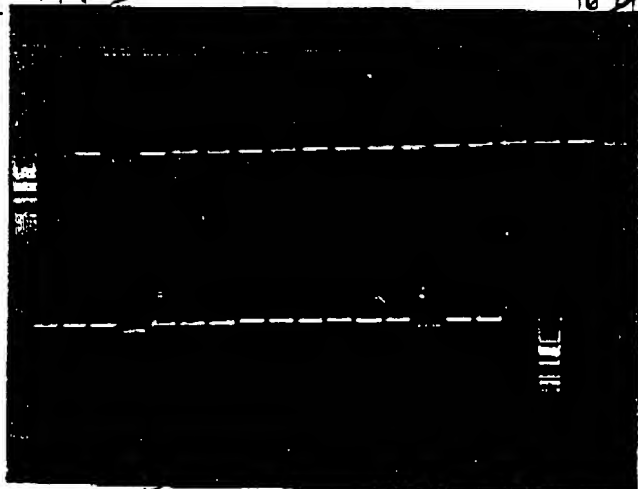
Restriction Digest BHC's

File	DNA	NGS #22	dt20	Language
1	BHG10-1 20.07	0.548	1	7.5
2	BHG10-2 20.07	6	2	1.1HindIII 2.4hr
3	BHG10-3 20.07	6	2	
4	BHG10-4 20.08	4	2	
5	BHG10-1 20.10	4	4	
6	BHG10-2 20.10	4	4	
7	BHG10-3 20.10	4	4	
8	BHG10-1 20.08	6	2	
9	BHG10-2 20.08	6	2	
10	BHG10-3 20.08	6	2	
11	BHG10-1 20.08	6	2	
12	BHG10-2 20.10	4	4	
13	BHG10-3 20.10	4	4	
14	BHG10-1 20.10	4	4	



1-33 15.106-0.6g #1 Sub Age1 7.0 1150

(parent) 1 2



BHG10-20.07-12 BHG10-20.10
seem to run differently pool
separately & redigest - dilute 20.07's
pool 11's & redigest

These clones look great nearly
all cut with Age1. have insert
#2, 17, 21 & 31 are insert - trash

I will further characterize 1 & 16
with XbaI
EcoRI
XbaI/EcoRI
Age1
XbaI/EcoRI/Age1
EcoRI/Age1
XbaI/Age1

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Witnessed & Understood by me,

Date

Invented by

Date

5/30/95

Recorded by

417KS